

Supplementary material: Pig production system drivers of antibiotic resistance in Uganda

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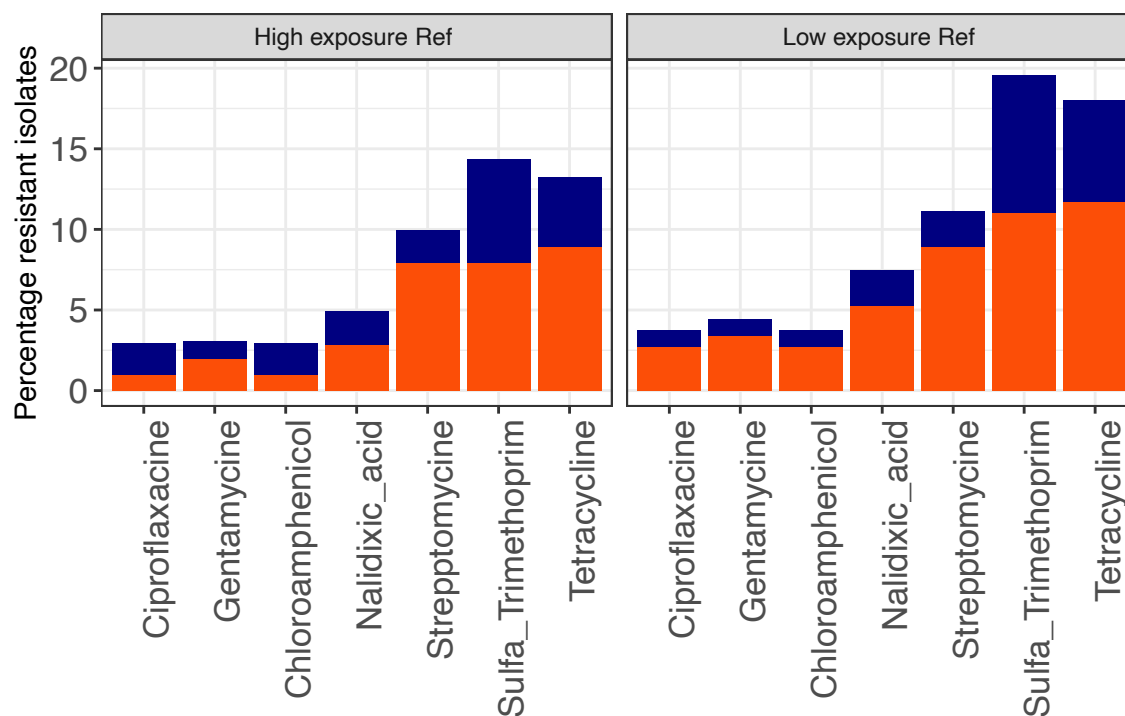
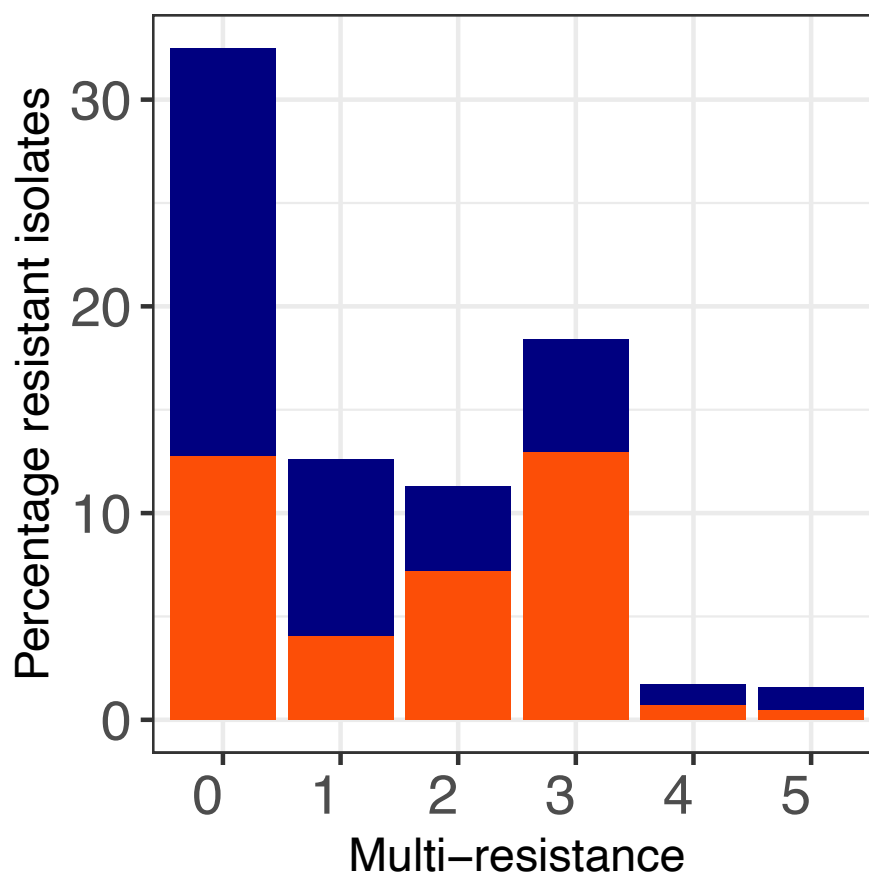
Extended Data Table S1. Summary of sampling and metadata collected from 70 households and 20 reference individuals during six visits in a one-year study of semi-intensive (peri-urban) and subsistence free-range (rural) pig production systems in Uganda. Metadata are presented according to the host types, i.e. farmers, exposure references and pigs. For farmers, we examined predictors such as gender, wealth attainment (marital status and education level), lifestyle-associated factors (smoking habits and alcohol consumption) and medication use. Data collected for pigs included pig breed, management-associated factors (housing, cleaning frequency and feed) and medication use. *On some visits, the farmer or exposure reference did not declare some information which we were, therefore, unable to record.

Variable	Categories	Household*	Pig production system* “Kampala and Mubende districts”		Sentinel bacteria
			Semi-intensive	Free-range*	
Farmer	Levels	Participants (N=70)	Faecal samples collected (N=386)		Sentinel bacteria (N=296)
Gender*	Female	22(31.4%)	56(46.0%)	65(44.0%)	100(33.7)
	Male	48(68.6%)	140(52.0%)	125(48.0%)	196(66.2%)
Marital status	Divorced	3(4.2%)	3(16.0%)	15(84.0%)	10(3.3%)
	Married	42(60%)	144(53.0%)	123(47.0%)	202(68.2)
	Single	14(20%)	40(50.0%)	40(50.0%)	66(22.2)
	Widow	4(5.7%)	9(42.0%)	12(58.0%)	15(5%)
Education	None	2(2.8%)	2(100.0%)	0(0.0%)	-
	Primary	27(38.5%)	74(50.3%)	73(49.7%)	112(37.8%)
	Secondary	31(44.3%)	94(54.3%)	79(45.7%)	143(48.3)
	Tertiary	10(14.2%)	26(40.6%)	38(59.4%)	38(12.8%)
Smoking status	Yes	3(4.3%)	0(0.0%)	17(100.0%)	15(5%)
	No	67(95.7%)	196(53.0 %)	173(47.0%)	281(95%)
Alcohol consumption status	Yes	20(28.5%)	59(53.1%)	52(46.9%)	219(74%)
	No	50(71.4%)	137(49.8%)	138(50.2%)	77(26%)
Antibiotic use	Yes	15(21.4%)	44(52.3%)	40(47.7%)	232(78.3%)
	No	55(78.5%)	152(50.3%)	150(49.7%)	64(21.7%)
Exposure references*		(N=20)	(N=109)		(N=76)
Gender*	Female	4(20%)	6(30%)	14(70.0%)	16(21%)
	Male	16(80%)	19(45.2%)	23(54.8%)	60(79%)
Marital status	Divorced	3(15%)	0(0.0%)	3(100.0%)	2(3%)
	Married	12(60%)	20(38.4%)	32(61.6%)	50(75.8)
	Single	3(15%)	5(100.0%)	0(0.0%)	11(16.6%)
	Window	2(10%)	0(0.0%)	2(100.0%)	3(4.6%)
Contact with pigs	Low	10(50%)	25(47.2%)	28(52.8%)	33(43.4%)
	High	10(50%)	29(51.7%)	27(48.3%)	46(56.6)
Education	None	2(10%)	2(100.0%)	0(0.0%)	3(4%)
	Primary	9(45%)	7(31.8%)	15(69.2%)	23(30%)
	Secondary	6(30%)	10(41.6%)	14(58.4%)	25(33%)
	Tertiary	3(15%)	6(42.8%)	8(57.2%)	15(20%)
Smoking status	Yes	2(10)	0(0.0%)	5(100.0%)	5(7%)
	No	18(90)	25(43.8%)	32(56.2%)	61(80%)
Alcohol consumption status	Yes	8(60%)	18(66.6%)	9(33.4%)	35(46%)

	No	12(60%)	7(20.0%)	28(80.0%)	31(41%)
Antibiotic Use	No				57(75%)
	Yes				9(12%)
Pigs		(N=70)	(N=382)	(N=296)	
Antibiotic use*	Yes	28(40%)	73(62.3%)	44(37.7%)	87(29%)
	No	42(60%)	113(48.9%)	118(51.1%)	180(61%)
Breed	Exotic	21(30%)	30(45.4%)	36(54.6)	55(19%)
	Local	14(20%)	42(56%)	33(44.0%)	31(10.4%)
	Mixed	35(50%)	114(55.1%)	93(44.9%)	171(58%)
Housing	Indoor	45(65%)	132(52.3%)	120(47.7%)	225(76.0%)
	Outdoor	25 (25%)	54(56.2%)	42(43.8%)	42(14.1%)
Feeding	Commercial feed	5(7.5%)	11(34.3%)	21(65.7%)	23(8%)
	Mixed	52(74%)	163(57.2%)	122(43.8%)	231(78%)
	Swill	13(18.5)	12(38.7%)	19(61.3%)	13(4.3)
Cleaning frequency	Daily	32(45.7%)	97(54.8%)	80(45.2)	163(55%)
	Twice a week	9(12.8)	30(49.1%)	31(50.9)	44(15%)
	Weekly	19(27.1)	28(56%)	22(44.0%)	35(12%)
	Never	10(14.4)	31(51.6%)	29(58.4%)	25(8.4%)

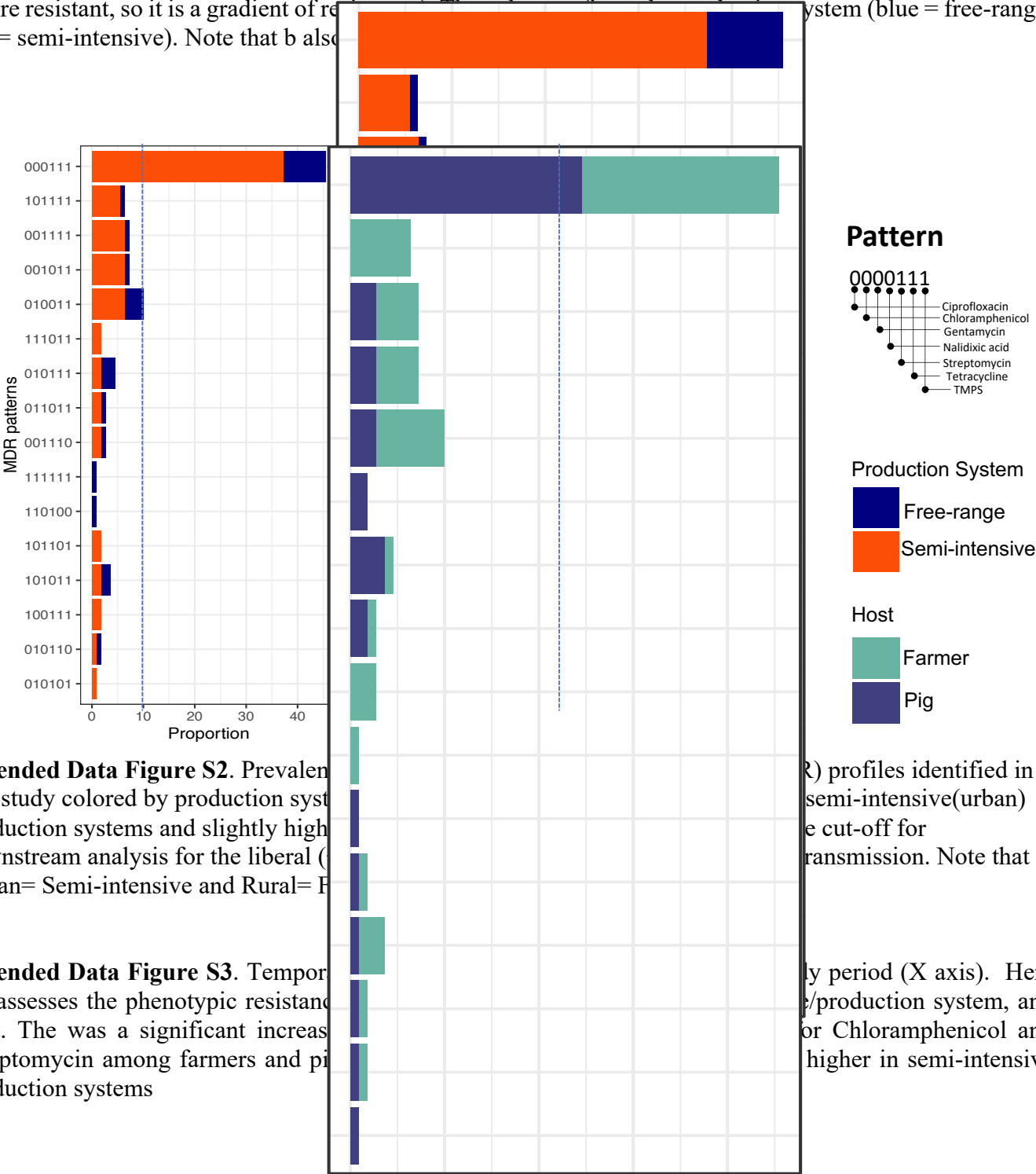
Phenotypic resistance of field exposure references

There were differences in the prevalence of phenotypic resistance between references low and high exposure references. The resistance was higher in semi-intensive production systems. The proportion of resistant sentinel bacteria was generally lower for exposure references in free-range production systems. This difference was wider between exposure references when examining resistance to streptomycin and tetracycline (Fig. S1). We considered a **high exposure reference** an individual who is in contact with pig gut content much more frequently than the average person, for example a person who is responsible for intestinal evisceration in an abattoir. In contrast to this type of exposure reference, a **low exposure reference** would be a person who has limited to no contact with pigs due religious beliefs. In general the proportion of resistance are lower than those in pigs and farmers

a**b**

Extended Data Figure S1. a Comparison of phenotypic resistance levels of high (individuals likely to come into contact with pig intestinal content) and low (individuals at very low risk of coming into contact with pigs) exposure references recruited to provide a comparison with study participants (farmer-pig pairs) in Uganda. The Y axis relates to the percentage of sentinel bacteria that were resistant to each of the antibiotics tested (X

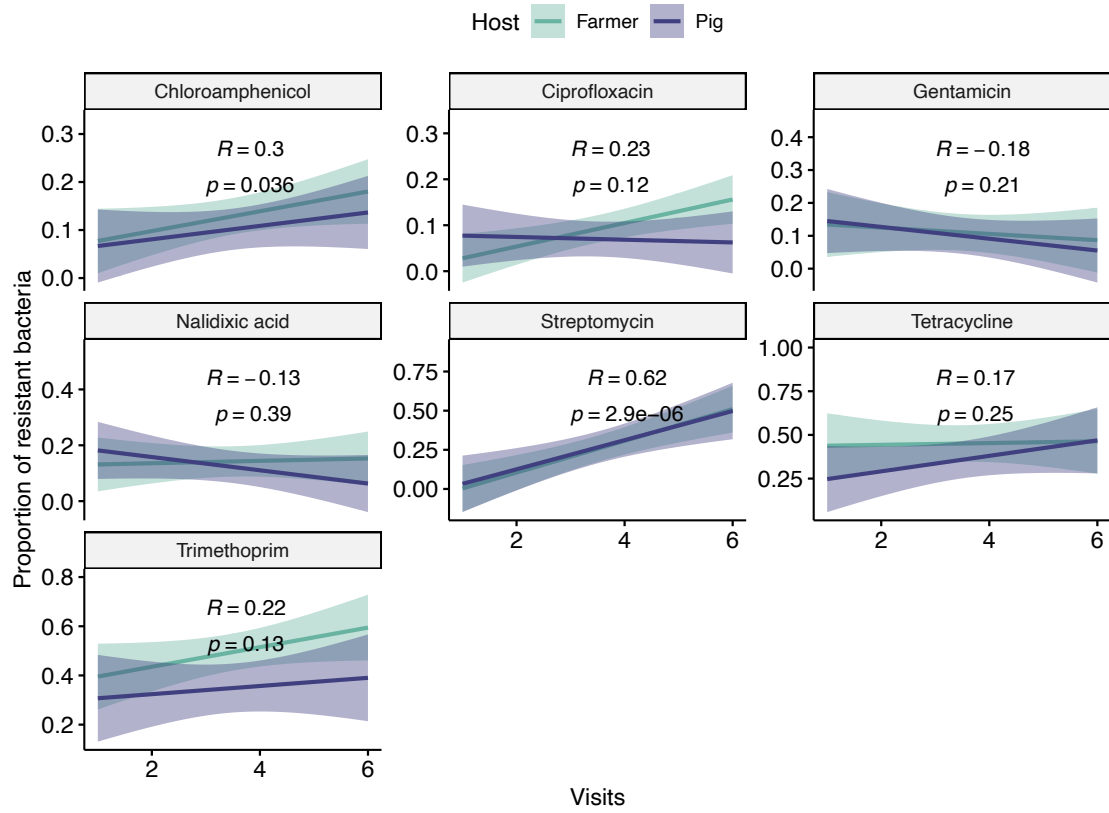
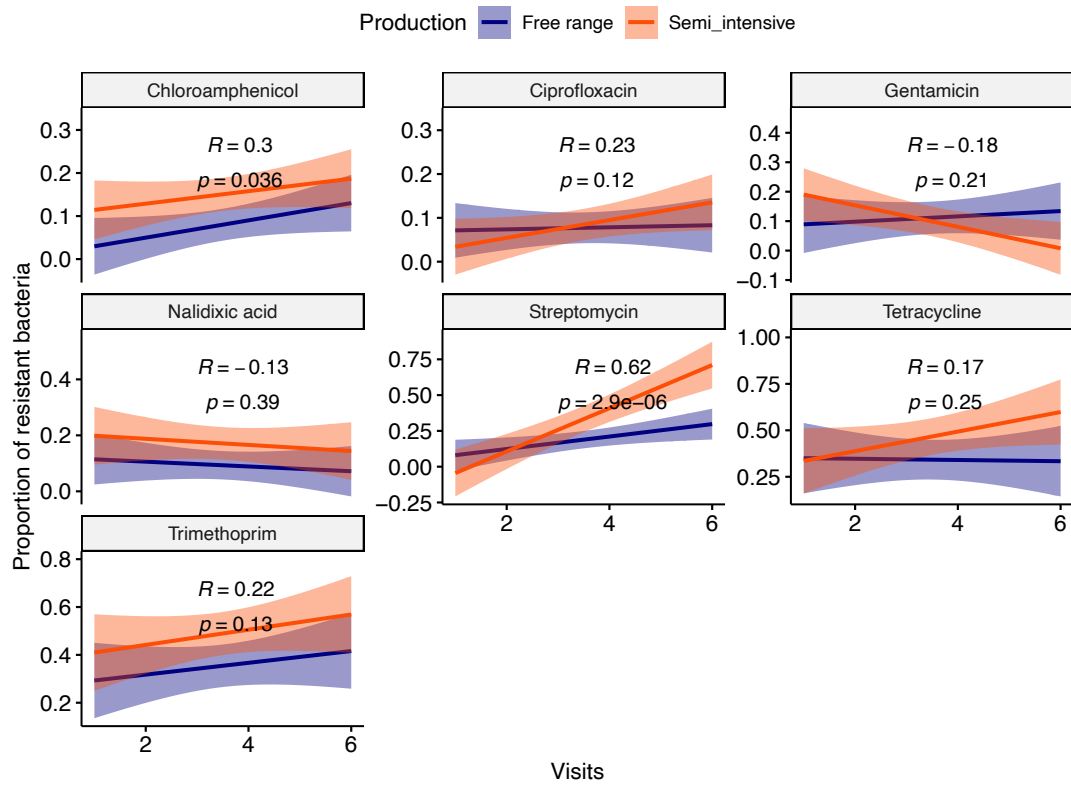
axis is the type of antibiotic. It is noteworthy that the proportion of antibiotic-resistant isolates obtained in these references is much lower than that of pigs and farmers sampled in this study. **b** Proportion of bacterial isolates (Y axis) that were resistant to multiple antibiotics (X axis = number of antibiotics to which bacteria were resistant, so it is a gradient of resistance). Note that b also



Extended Data Figure S2. Prevalence of MDR profiles identified in this study colored by production system (blue = free-range; orange = semi-intensive) and slightly higher proportion of isolates resistant to multiple antibiotics (X axis = number of antibiotics to which bacteria were resistant, so it is a gradient of resistance). Note that b also

Extended Data Figure S3. Temporal trends in the proportion of MDR profiles identified in this study colored by production system (blue = free-range; orange = semi-intensive) and slightly higher proportion of isolates resistant to multiple antibiotics (X axis = number of antibiotics to which bacteria were resistant, so it is a gradient of resistance). Note that b also

R) profiles identified in semi-intensive(urban) production systems and slightly higher proportion of isolates resistant to multiple antibiotics (X axis = number of antibiotics to which bacteria were resistant, so it is a gradient of resistance). Note that b also

a**b**

Extended Data Table S2. Mixed-effect logistic regression model to identify factors associated with phenotypic resistance of *Escherichia coli* and *Klebsiella* species isolated from farmer-pig pairs in semi-intensive and free-range production systems of Uganda.

Variables	Levels	Odds ratios (95%CI)	P Value	Variance
Fixed effects				
Production system	Semi-intensive	1 (ref.)		
	Free-range	1.00 (0.66-1.51)	0.996	
Sentinel bacteria	<i>E. coli</i>	1 (ref.)		
	<i>Klebsiella</i>	0.42 (0.32-0.55)	P<0.001	
Pig breed	Exotic	1 (ref.)		
	Local	0.44 (0.24-1.81)	0.009	
	Mixed breed	0.86 (0.60-1.23)	0.41	
Antibiotic	Ciprofloxacin	1 (ref.)		
	Gentamycin	1.29 (0.73-2.30)	0.38	
	Nalidixic acid	2.55 (1.51-4.32)	P<0.001	
	Streptomycin	7.02 (4.27-11.55)	P<0.001	
	Trimethoprim/sulfamethoxazole	13.78 (8.39-22.64)	P<0.001	
	Tetracycline	13.17(8.03-21.62)	P<0.001	
Temporal scale	Visits	1.14 (1.03-1.27)	0.014	
AMR gene levels	<i>tetQ</i>	1.29 (1.11-1.51)	0.001	-
Interaction term	Semi-intensive*pig	0.56(0.31-1.02)	0.06	
Random effects				
Farm ID				0.17
Visit				0.01

Extended Data Table S3: Mixed-effect logistic regression model examining the relationship between phenotypic resistance to six antibiotics of sentinel bacteria *Escherichia coli* and *Klebsiella* isolated from farmers, pigs and high (individuals likely to come into contact with pig intestinal content) and low (individuals at very low risk of coming into contact with pigs) exposure references (HE Ref and LE Ref, respectively) in semi-intensive and free-range production systems of Uganda.

Predictors	Phenotypic resistance estimates		
	Odds Ratios	CI	p value
Production [Semi-intensive]	1.42	1.00 – 2.01	0.050
Bacteria [<i>Klebsiella</i>]	0.5	0.41 – 0.59	<0.001
Host [LE Ref]	0.54	0.29 – 1.03	0.060
Host [Pig]	0.50	0.35 – 0.70	<0.001
Host [HE Ref]	0.41	0.21 – 0.81	0.010
Antibiotic [gentamicin]	1.47	0.98 – 2.22	0.063
Antibiotic [nalidixic acid]	2.35	1.60 – 3.46	<0.001
Antibiotic [streptomycin]	6.04	4.20 – 8.69	<0.001
Antibiotic [Trimethoprim/sulfamethoxazole]	12.21	8.51 – 17.50	<0.001
Antibiotic [tetracycline]	11.53	8.04 – 16.54	<0.001
Semi-intensive: LE Ref	2.20	0.97 – 4.98	0.058

Semi-intensive: Pig	1.67	1.11 – 2.51	0.015
Semi-intensive: HE Ref	1.77	0.78 – 4.00	0.169
Random Effects			
σ^2	3.29		
τ_{00} household_id	0.20		
τ_{00} TIMEZ	0.10		
ICC	0.08		
N _{household id}	136		
N _{TIMEZ}	6		
Observations	3388		
Marginal R ² / Conditional R ²	0.265 / 0.326		

Factors associated with ABR gene and phenotypic resistance with in exposure references included in the statistical models

Phenotypic resistance estimates ranged from 4% to 60% for ciprofloxacin and sulfamethoxazole/trimethoprim, respectively. Resistance was highest and lowest in bacteria isolated from farmers and high and low exposure references, respectively. Farmers had higher gene carriage than the exposure references but generally pigs had fewer genes than farmers and exposure references.

Our assumption was that the sharing of multidrug resistance (MDR) traits is a rare event at the household level and even more rare between pigs in different sub-counties, and districts. Therefore, when encountered it likely represents a transmission event. We used sixteen unique MDR patterns to investigate this question. The most common MDR pattern was resistance to streptomycin, tetracycline and trimethoprim/sulfamethoxazole which are the most used antibiotics in these livestock production systems.

ABR gene carriage for the exposure reference groups

Extended Data Table S4: Mixed-effect linear regression model examining the relationship between gene carriage among host (i.e. farmer/pig), and high (individuals likely to come into contact with pig intestinal content) and low (individuals at very low risk of coming into contact with pigs) exposure references (HE Ref and LE Ref, respectively), in semi-intensive and free-range production systems of Uganda.

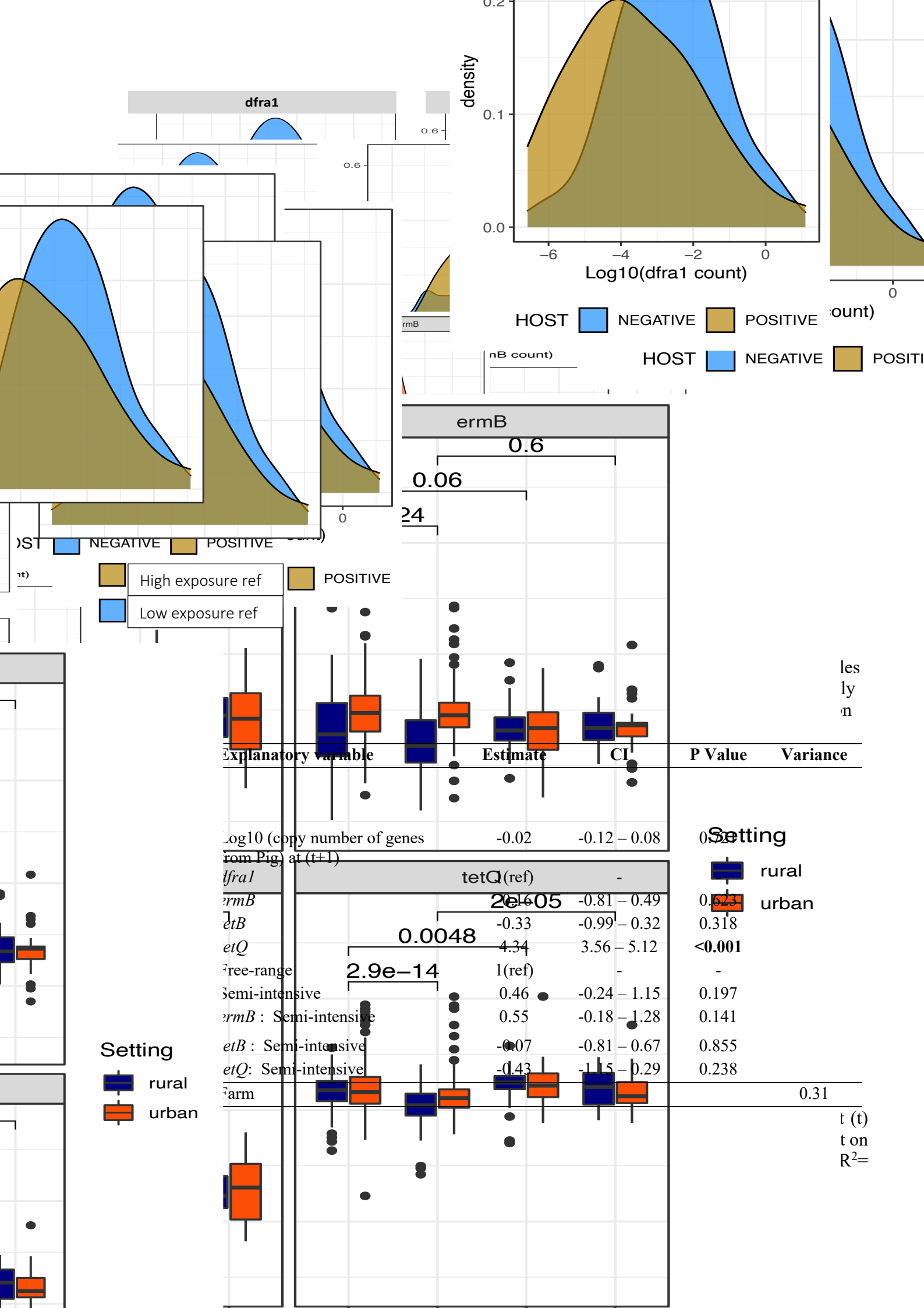
<i>Predictors</i>	log10(AMR gene copy number)		
	<i>Estimates</i>	<i>95% CI</i>	<i>p value</i>
Host [Pig]	0.02	-0.09 – 0.12	0.768
Host [HE Ref]	1.89	1.24 – 2.53	<0.001
Host [LE Ref]	1.76	1.12 – 2.41	<0.001
Time	0.04	-0.18 – 0.26	0.738

Genes [<i>ermB</i>]	-0.47	-0.58 – -0.36	<0.001
Genes [<i>tetB</i>]	-0.56	-0.58 – -0.35	<0.001
Genes [<i>dfra</i>]	-1.55	-1.78 – -1.31	<0.001
Production [Semi-intensive]	0.78	0.44 – 1.12	<0.001
Pig: Semi-intensive	-0.02	-0.15 – 0.11	0.737
HE Ref: Semi-intensive	-0.90	-1.74 – -0.07	0.034
<i>ermB</i> : Semi-intensive	0.12	-0.02 – 0.26	0.087
<i>tetB</i> : Semi-intensive	0.35	0.21 – 0.48	<0.001
<i>dfra1</i> : Semi-intensive	0.24	-0.05 – 0.54	0.108

Random Effects

σ^2	1.97
$\tau_{00 \text{ hh_id}}$	0.43
$\tau_{00 \text{ TIME}}$	0.22
ICC	0.25
$N_{\text{hh_id}}$	85
N_{TIME}	6
Observations	11039
Marginal R^2 / Conditional R^2	0.122 / 0.338

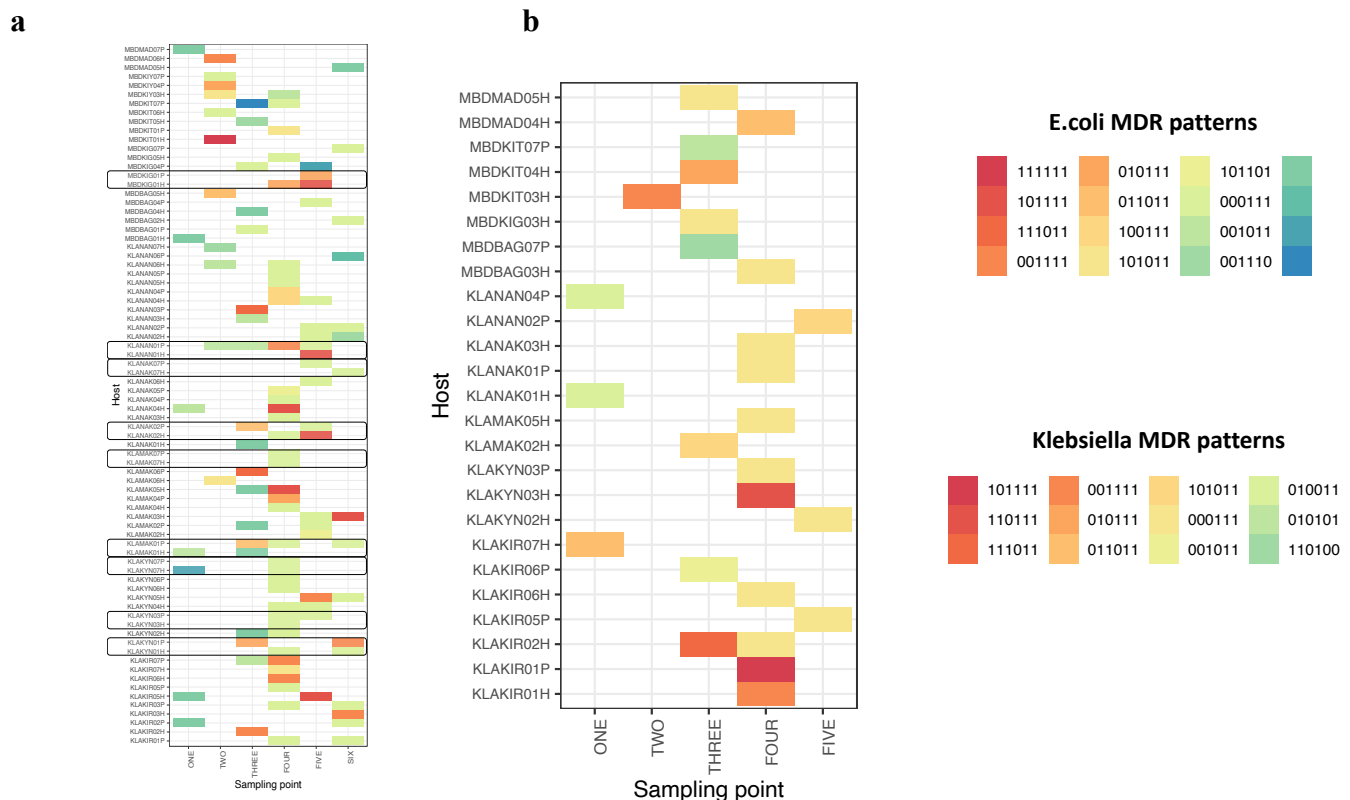
There were slight differences between gene carriage by farmer and pig in semi-intensive and free-range systems. However, we observed significant differences between ABR genes carriage by pigs and exposure references. This was the case for all genes, with an overall higher carriage of AMR genes in semi-intensive systems.



Extended Data Table S6. Mixed-effect linear regression model to identify factors associated with gene carriage in farmers and their pigs in semi- intensive and free-range production systems of Uganda. ABR = antibiotic resistance.

Variables	Levels	Estimate (95%CI)	P Value	Variance
Fixed effects				
Log10(Gene copy number)	Intercept	-3.32 (-3.71- -2.94)	P<0.001	
Production	Semi-intensive	1 (ref.)		
	Free-range	0.49 (0.25-0.72)	P<0.001	
Host	Farmer	1 (ref.)		
	Pig	-0.64 (-0.84- -0.45)	P<0.001	
ABR genes	<i>dfra1</i>	1 (ref.)		
	<i>ermB</i>	0.29 (0.14-0.45)	P<0.001	
	<i>tetB</i>	-0.72 (-0.87-0.56)	P<0.001	
	<i>tetQ</i>	3.97 (3.81-4.12)	P<0.001	
Interaction term	Semi-intensive*pig	0.27(0.02-0.51)	0.031	-
Random effects				
Farm				0.10
Visit				0.16

Inference of transmission at the human animal interface



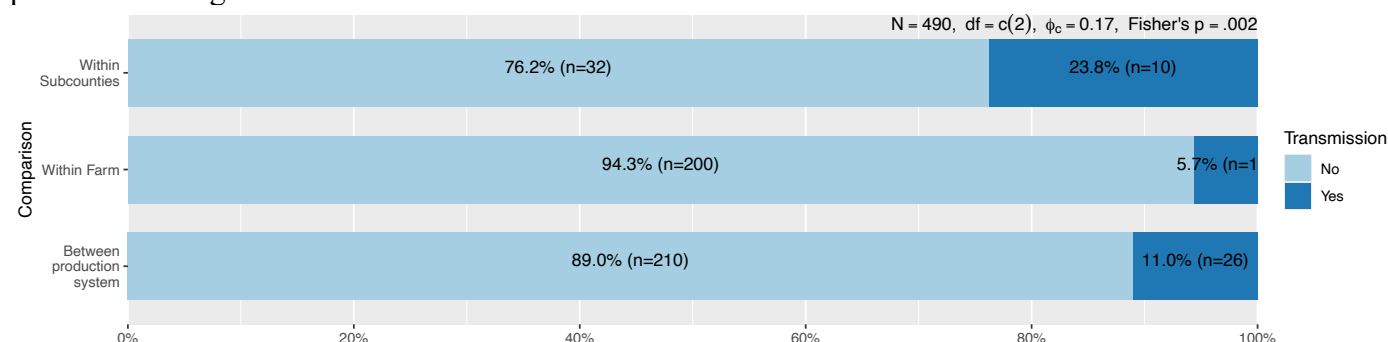
Extended Data Figure S7: Tile plot showing the carriage of multidrug resistance (MDR) profiles at the household/farm level over time. **a** Sharing of events for *Escherichia coli* and **b** for *Klebsiella* spp. The color coding represents a gradient of MDR, with blue indicating the smallest number of antibiotics to which isolated bacteria (isolate identification number is provided on the Y axis) were resistant to and red the highest number.

Extended Data Table S7: The proportion of shared MDR strains between a farmer and their pig at farm level, and within and between production systems.

Comparison	Transmission estimates	
	Conservative N=490	Liberal N=1850
Farmers and pigs between production systems	(26)11%	(66)11.1 %
Farmers and pigs within production systems	(12)5.7 %	(330)30.7%
Farmers and pigs on farms	(10)23.8%	(68)38.2 %
Prevalence of transmission events	(48) 9.8%	(464) 25.1%

$\chi^2=13.836 \cdot df=2 \cdot \text{Cramer's } V=0.168 \cdot \text{Fisher's } p=0.001$ (Conservative estimates)
 $\chi^2=96.422 \cdot df=2 \cdot \text{Cramer's } V=0.228 \cdot p=0.000$ (Liberal estimates). Note here that we have used the liberal estimate for the figure 6A

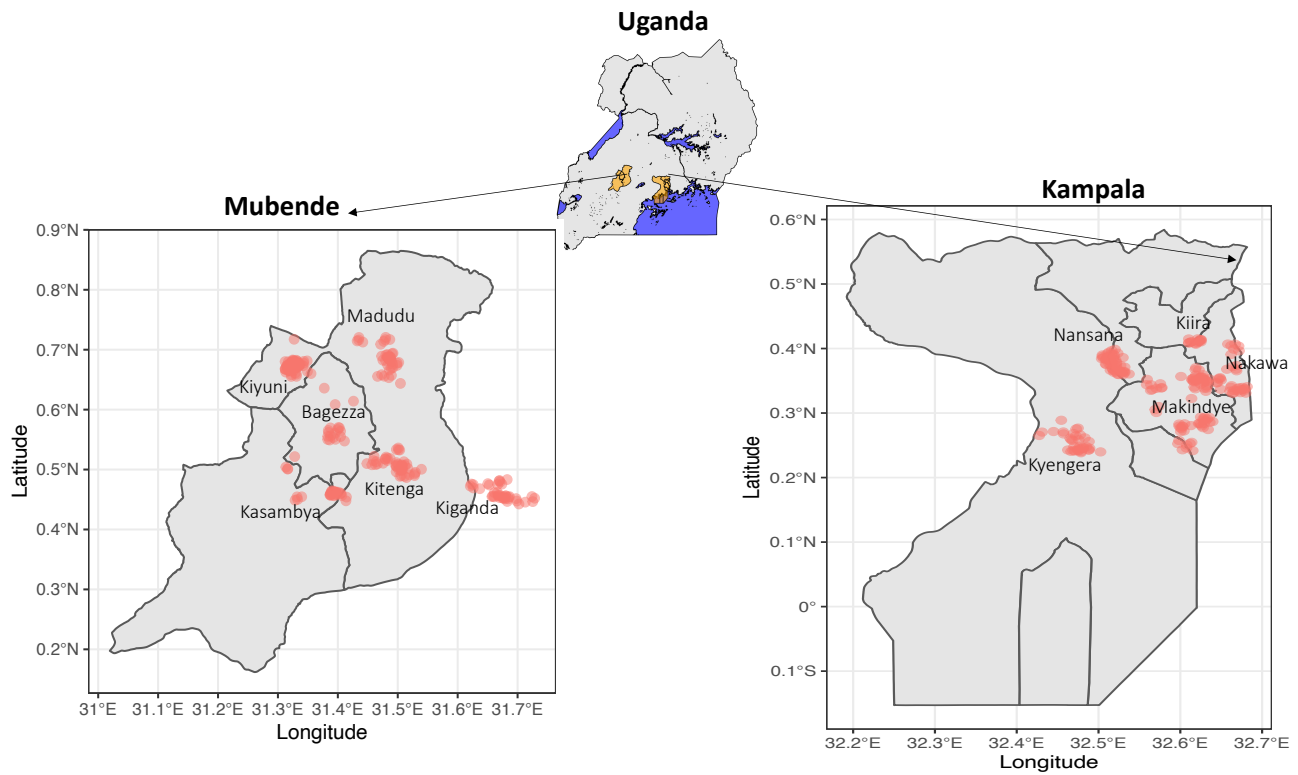
Extended Data Figure S8. The frequency of transmission events at different spatial scales, farm/household, within and between production systems. This figure relates to Extended Data Table S7 above it shows the prevalence using the conservative estimates



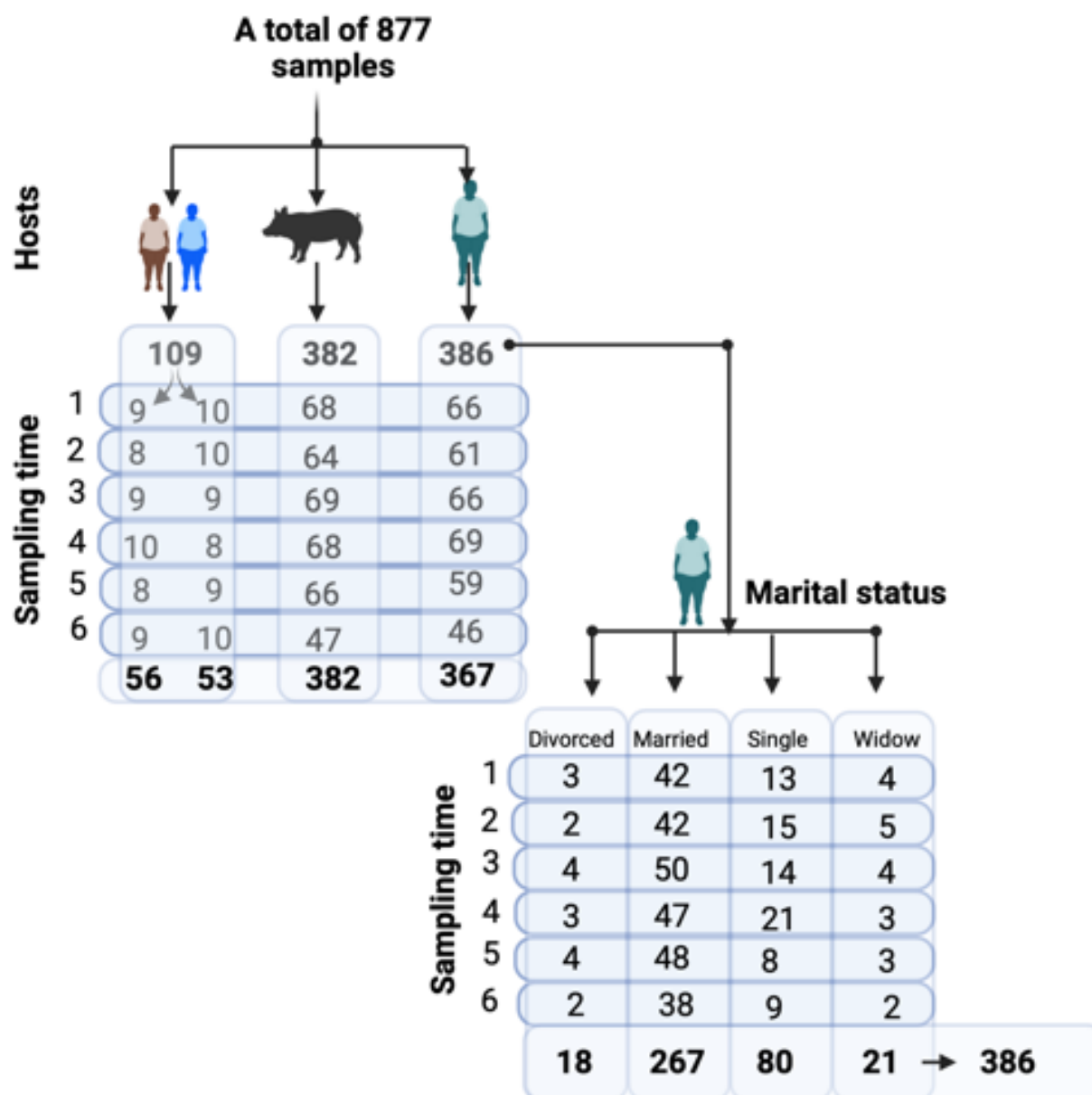
Materials and Methods

Study site

This study was conducted in two districts of Uganda, Kampala and Mubende, - five sub-counties each - representing peri-urban (semi-intensive) and rural (/free-range) livestock production systems, respectively.



Extended Data Figure S9. Map of the study area showing the five sub-counties in each of the peri-urban (semi-intensive livestock production systems) and rural (free-range livestock production systems) districts (Kampala and Mubende, respectively) of Uganda where samples and data analysed in this study were collected from pairs of pigs and farmers. The pink bubbles represent all the visits to a household during the study jittered at 0.001×0.001 for data governance reasons.



Extended Data Figure S10: Participant recruitment and sample collection across time- The flow chart shows the number of study participants recruited in this study, as well as the samples collected from each group at each of the six sampling points and marital status information for the farmers interviewed. We illustrate host and marital status information available for each of the farms recruited in order to elucidate any disparities in numbers that arose due to missing data. For instance, while marital status information was available for all 386 farmers from whom samples were collected, the host label was missing for 19 sample, resulting in a discrepancy. The figure also displays the samples collected across these variables for the six sampling points, facilitating an evaluation of the temporal aspects in this longitudinal cohort study. This aims to highlight the discrepancies that can arise due to missingness in data.

Phenotypic antibiotic resistance diffusion disc interpretation

Disc diameters were interpreted using the European Committee on Antimicrobial Susceptibility Testing system ([EUCAST](#)) to assign a status of “S”, “I” and “R” to indicate Susceptible, Intermediate and Resistant sentinel bacteria, respectively. We then extracted an aliquot of high molecular grade DNA for downstream analyses.

DNA extraction and quantification

DNA extraction was performed using the Ultraclean Soil DNA Isolation Kit (MO BIO Laboratories). DNA quantification and quality checks were conducted on each sample extract using the Qubit fluorometer. Antibiotic resistance gene copy numbers were normalised using the 16S rRNA gene copy number which was used as a proxy for the number of bacterial cells per sample. This experiment was done using our recently published in-house methodology¹. The gene count was normalised by dividing the gene copy number of each gene by the total bacterial load in each sample. So, in effect, this outcome variable approximates the gene copy number per bacterium.

References

1. Pollock J, Muwonge A, Hutchings MR, et al. Resistance to change: AMR gene dynamics on a commercial pig farm with high antimicrobial usage. *Sci Rep* 2020; **10**: 1708.